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Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

for

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

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Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Entered as second-class matter at the Post Office at Baltimore, Md.

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NUTRITION REVIEWS

VOL. 16 SEPTEMBER 1958 No. 9

NUTRITION AND BEHAVIOR

In countries with adequate facilities for food production and distribution, the make-up of the diet depends largely on the consumer's choice. The most nutritious cooked breakfast cereal will do no good, if it is not consumed on account of an unacceptable flavor. The appraisal of flavor is a task involving consideration of behavior.

Some indication of the breadth and current literature on this topic is given by the following sources: A survey (D. R. Peryam, F. J. Pilgrim, and M. S. Peterson, editors, *Food Acceptance Testing Methodology, Nat. Res. Council, Washington, D. C. (1954)*), sponsored by the Quartermaster Food and Container Institute for the Armed Forces, Chicago; a record of a conference held in New York at the Academy of Sciences (A. Turk, editors, *Basic Odor Research Correlation, Ann. N. Y. Acad. Sci.* **58,** *Article 2, (1955)*); and a review of the "profile method" of food analysis (J. F. Caul, *Adv. Food Res.* **7,** *1 (1956)*).

The significance of cultural factors in nutrition studies was brought out by E. Wellin (*Nutrition Reviews* **13,** *129 (1955)*). Food habits are thoroughly immersed in culture and they affect food intake as importantly as do the soil, the climate, and the level of economic and agricultural development. Wellin (*loc. cit.*) noted that ethnographic accounts may identify important "natural" experiments on nutrition. Culturally conditioned differences were put to study by a group of investigators in the far away Bosnia-Hercegovina. They contrasted two groups of the same national origin (Serbo-Croatian) but which differed in religion (G. Žarković, M. Levi, M. Radovanović and T. Plećaš, *Acta Med. Jugoslav.* **9,** *129 (1955)*). The Orthodox (Serbs) tended to have a higher dietary fat intake (and a higher serum cholesterol level) than the Moslems. Here we have the sequence: 1) cultural determinants, 2) dietary intake, and 3) body metabolism.

However, one can turn around and cite dramatic effects of nutrition (specifically of diets defective in composition or deficient in amount) upon behavior. In famine (P. A. Sorokin, *Man and Society in Calamity*, E. P. Dutton, New York (1942)), and under experimental conditions (A. Keys, *et al., The Biology of Human Starvation*, Univ. of Minnesota Press (1950)) man's behavior and work capacity are profoundly altered. It is to J. Goldberger's credit (*J. Am. Med. Assn.* **66,** *471 (1916)*) to have discovered that the psychosis, which constitutes an important component of the pellagra syndrome, is a consequence of a dietary deficiency, a behavioral manifestation of a disordered brain metabolism, amenable to a specific dietary treatment (*See* H. Sebrell, *Res. Publ. Assoc. Nerv. Ment. Dis.* **22,** *113 (1943)*).

Is there, in our own days, a more dramatic development in the therapy of mental deficiency than the hope for a control of phenylpyruvic oligophrenia (H. McIlwain, *Biochemistry and the Central Nervous System*, Little, Brown, and Co., Boston, pp. 115 (1955)) through dietary means? In this case we do not deal with a dietary deficiency but rather with a faulty metabolism, involving the inability of the young child to metabolize normally the amino acid phenylalanine. It is converted into phenylpyruvic acid, which the patients are also unable to oxidize. By maintaining children affected by this metabolic abnormality on diets low in phenylalanine during the first years of life, great improvement in mental functioning has been obtained (H. Bickel, J. Gerrard, and E. M. Hickmans, *Lancet* **II,** *812 (1953)*; H. Bickel, *Exp. Med. Surg.* **12,** *114 (1954)*; L. I. Woolf, R. Griffiths, and A. Monerief, *Brit. Med. J.* **1,** *57 (1955)*).

Apathy and anorexia were repeatedly noted among the clinical characteristics of severe protein malnutrition, varying in name but severely affecting children in many parts

of the globe. The behavioral manifestations are being examined using standardized psychological test situations and evaluative procedures at the Hospital Infantil at Mexico City (F. S. Gomez *et al.*, *Bol. Méd. Hosp. Infantil* **11,** *631 (1954)*) where the clinical investigators study the *sindrome pluricarencial de la infancia*, and at Kampala, Uganda, where M. Geber and R. F. A. Dean (*UNESCO Courrier* **6,** *3 (1956)*) examine the psychological changes accompanying kwashiorkor.

A symposium at the Laboratory of Physiological Hygiene, under the sponsorship of the School of Public Health, University of Minnesota, dealt with some facets of the manifold relationships between nutrition and behavior (*Am. J. Clin. Nut.* **5,** *no. 2 (1957)*).

There were four sections in the symposium, concerned with: 1) Impact of nutrition on behavior; 2) hunger and appetite; 3) food appraisal and acceptance by man; and 4) satiety and weight control.

The symposium presents an informative picture of recent developments in the complex field of studies on nutrition and behavior. (A reprint of the proceedings, together with an added epilogue and an index, is available in book form from the National Vitamin Foundation, Inc., New York.)

What of the future? Commercially, flavor control is as essential to the standardization of many products as is the food composition, and the problems of food flavor and food acceptance will remain of interest to the food industry, the Armed Forces, the Department of Agriculture, and scientists in academic institutions. The relation between chemical composition and the sensory effects of chemical compounds is inadequately understood at the present time.

Food-directed behavior involves much more than physiologic need. It includes appetite, esthetic enjoyments, and remote goals, including longevity. Thus, the behavior of organisms toward food provides a useful information model for the general study of the dynamics of human behavior. More extensive use of the psychologist's tools for a quantitative study of sensory functions, motor performance, intelligence, and personality will contribute to a more comprehensive evaluation of the significance of nutrition in man's biology.

Controlled investigations indicate clearly that deficits of calories and of essential nutrients, such as some components of the vitamin B complex, have a profound impact upon both components of work performance, i.e. work capacity and motivation. The degree and nature of adaptation to undernutrition and malnutrition, with special reference to the functioning of the endocrines and of the nervous system, and the impact of improved nutrition in the underdeveloped parts of the world represent some of the most intriguing research topics in human nutrition.

In large parts of the world the crucial problem is not the combat of frank nutritional deficiencies, but rather the development and popular acceptance of a diet conducive to maximal health and vigorous longevity. In the combat of degenerative heart disease, in importance far outstripping all other causes of death in western civilization, weight control and diet loom large as promising leads in prevention. Weight reduction, whether undertaken as a preventive or a curative measure on an individual or a group basis, is a fruitful field of research and application for the clinical psychologist, the psychiatrist, and the social psychologist, working in cooperation with nutritionists or internists.

In the light of accumulating evidence it may be that food habits, and the attempts to modify them, will become one of the crucial topics in the study of the relationships between nutrition and behavior in the next phase of the development of the science of human nutrition.

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TREATMENT AND PREVENTION OF KWASHIORKOR

Protein deficiency constitutes one of the most serious health problems of undeveloped nations. Growing children especially feel the effects of this, usually in the form of kwashiorkor (*Nutrition Reviews* **15,** *101, 132, 169, 199, 207, 254, 264 (1957)*; **16,** *36, (1958)*). The symptoms of this disease include edema, lesions of the skin and mucous membranes, changes in the hair, hypoproteinemia, lowering of the blood amylase level, and fatty degeneration of the liver. These conditions can be followed by malfunction of the pancreas and the liver, which lay the foundation for more serious complaints in adult life. Treatment of kwashiorkor by a diet rich in protein may give excellent results. However, the most effective treatment must be preventative and should be based on education of the population and increased production and utilization of inexpensive protein.

J. Sénécal (*Ann. N. Y. Acad. Sci.* **69,** *916 (1958)*) has studied this deficiency, as it exists in French West Africa, from three very enlightening perspectives. In the first place he has experimented with the forms in which protein may be most easily utilized by the subject already suffering from kwashiorkor. Second, he has studied the biological and biochemical alterations in such subjects, and, third, he has made recommendations of protein sources which are at the same time nutritionally adequate and available.

From a consideration of the possibility that the exocrine cells of the pancreas may have suffered early and serious damage, it seemed advisable to try protein hydrolyzates in the protein-deficient patient. When an acid hydrolyzate and an enzyme hydrolyzate of casein and lactalbumin were given orally (8 to 12 g. per kg. per day), more rapid results were obtained than with milk alone and, even more markedly, than with vegetable protein. Edema disappeared in three to four days and skin lesions were gone in ten days. However, toward the fifth day, some patients suddenly fell into a deep coma and died. Serum analysis showed a significant decrease in blood glucose levels which appeared to be the source of the trouble. Subsequently, a diet much richer in carbohydrates was provided along with the protein hydrolyzate. The best levels of nitrogen retention were obtained when the components of the diet (carbohydrates, fats and proteins) reached a balance in which the proteins constituted 25 per cent of the total calories. With this diet, nitrogen balance remained strongly positive for several weeks and nitrogen retention reached as much as 50 to 60 per cent by the fifteenth day. Cheilosis, which was usually present, was not cured by vitamins B_1 and B_{12} and niacin, whereas a high-protein diet caused it to disappear within a few days. Since blood potassium levels in patients at the time of admission were often low because of diarrhea and vomiting plus the existing anorexia, potassium was given in large doses (100 to 200 mg. per kg. per day). This was especially required for tissue repair.

Along with the better known clinical symptoms of kwaskiorkor, Sénécal found that total serum proteins in protein-deficient patients were low when analyzed electrophoretically. The albumin:globulin ratio was low and there was an increase in $alpha_2$ protein. During high protein treatment, the $alpha_2$ protein decreased, the albumin increased and, in the cured patients, the albumin:globulin ratio was approximately 1, as in the normal patient. Amino acid chromatograms of the blood and urine of children suffering from kwashiorkor show more numerous spots, which stain more intensely than do those of normal children. Alanine, glycine and serine appear constantly to be present in the urine, and leucine, valine, proline, tyrosine and threonine are frequently observed. Lysine, aspartic acid and phenylalanine are not detected in the urine when this technique is used.

In considering possible available sources of protein in French West Africa, so that a positive educational program could be carried out, Sénécal discounted both local milk production and importation of dry milk. Greatly hampered by poor pasture land, animal disease and difficulties of transportation, it would take many years to establish a milk industry. Dry milk is too expensive to be used extensively by the native population. In French West Africa millet or sorghum form the basic diet, with corn as a secondary foodstuff. Apart from these cereals, two protein sources exist that have not been exploited, peanut press cake (a by-product of oil production) and fish. The development of the latter source of proteins is very attractive since it can supplement the diet more effectively with the essential amino acids than can vegetable protein.

In trials conducted with children, Sénécal was able to ascertain which combinations of these available proteins are the most acceptable. Four kinds of supplementation were studied simultaneously in four groups of children in the clinic. The first group was a control in which the children were given supplementary calories (208) but no additional proteins. Group two received millet plus peanut press cake (227 calories). Group three received millet plus peanut press cake and fish flour (227 calories). The fourth group was also a control, and received the supplementary calories with the addition of 16.5 g. of milk protein (206 calories). Millet-peanut and millet-peanut-fish flour mixtures were definitely superior to millet alone. The results obtained with skim milk were surprising, as gain in weight was less than that obtained with the mixtures. The children in this last group, however, were somewhat younger (average of 18 months) than those in the other groups (average of 22 months).

In another series of experiments it was shown by the addition of amino acids, singly or in groups, that lysine had a limited effect in alleviating cases of acute kwashiorkor. However, at the present time individual amino acid metabolism is so obscure in this condition that the specific deficiency has not been determined.

Another group of workers in this field of nutrition, M. Béhar and co-workers (*Ann. N. Y. Acad. Sci.* **69,** *969 (1958)*), made observations on children suffering from kwashiorkor in Central America. They stress the role of infections in precipitating cases of kwashiorkor, especially those involving diarrhea. Diarrhea not only limits the absorption of nitrogen, but also considerably increases its excretion, so that the net retention drops. The situation is aggravated by the tendency of the mothers to withdraw solid food from the child who develops diarrhea and to substitute gruels high in starch content. The authors do not adhere to the recommendations of Sénécal (*loc. cit.*) that protein hydrolyzates or specific amino acids be used as supplementation. Their opinion seems to be that alleviation of the protein deficiency is readily enough achieved by a high natural protein diet. However, they present much less experimental evidence than did Sénécal to support their case. They are in general agreement with Sénécal and others regarding the inadequacy of vitamin therapy in treating this symptom of protein deficiency. Because of the prevalence of disease in their subjects, they do favor the use of antibiotics as penicillin during the first eight to ten days of hospitalization. Furthermore, the administration of potassium is recommended.

Since, in Central America, sources of animal protein are scarce and too expensive for the general population, these workers, under the auspices of the Institute of Nutrition of Central America and Panama, have developed a vegetable mixture now

under study in general field trials. It includes dried corn, 50 per cent; sesame meal, 35 per cent; cottonseed press cake, 9 per cent; torula yeast, 3 per cent; and kikuyu leaf meal, 3 per cent. The mixture contains adequate proportions of most of the essential amino acids per gram of nitrogen. Slight deficiencies of tryptophan, methionine, lysine and valine are present, however, and rats and baby chicks require additional lysine. To date, 11 children have received this mixture. The results have been good, no complications have developed during a 100-day interval, and the average retention was the same as that per equivalent weight of milk protein.

THE ROLE OF BREAST FEEDING IN IMMUNITY

The human infant derives a great majority of his initial immunity by the transfer of maternal antibodies across the placenta. The presence of antibody globulins in human colostrum (F. Nordbring, *Acta Pediat.* **46,** *482 (1957)*), and the observation that the colostrum is an important mechanism for the transfer of antibodies to newborn ungulates, raises the question of the importance, if any, of colostrum and mature milk in the development of immunity in the young human infant. Current interest in this subject is reflected in recent editorial commentary (*Pediatrics* **21,** *343 (1958)*).

Recent observations in cattle have demonstrated the presence of "diathelic" immunization in this species (B. Campbell, M. Sarwar and W. E. Peterson, *Science* **125,** *932 (1957)*). These authors have demonstrated that the organisms normally present in the mouth of the newborn calf have the ability of provoking the secretion of specific antibody by the udder during a period of only a few hours. This mechanism would seem to be a very important one in this species where transplacental passive immunity is relatively unimportant. As yet, no observations on the ability of the human breast to produce antibodies under similar circumstances have been made.

The human infant ingests antibodies during the period of colostrum feeding and may ingest smaller amounts with mature human milk. The passive immunity so acquired could be considered to be of clinical significance if it could be demonstrated to be associated with both antibody titers generally considered to be of clinical significance in these specific infections and a lower incidence of such infection in infants receiving human milk.

A large literature has accumulated concerning the morbidity and mortality from infectious diseases in artificially fed and breast fed infants. The more recent of these studies would suggest that artificial feeding by intelligent mothers with adequate medical supervision presented no hazard to their infants. There is only limited evidence suggesting a protective effect of breast milk in human infants. A large scale study in Britain (J. W. B. Douglass, *J. Obstet. Gynec. British Empire* **57,** *335 (1950)*) based on 4669 infants born in March, 1946, and followed for a two-year period revealed no mortality differences between artificially fed and breast fed infants.

The one striking finding in this study was that the incidence of measles in the breast fed infants was significantly lower than that in the artificially fed infants, and that this difference was striking only in the 10- to 24-month-old infants. The incidence of measles during the nursing period was not greatly different between the two groups. There was no difference in the incidence of whooping cough, German measles, or chicken pox in the same group,

and the diminished frequency of measles was seen in the highest social class as well as among the less privileged children. Although this observation is not documented in terms of protective levels of measles antibody it can only be interpreted as evidence that breast feeding for three months or longer may have a significant effect upon certain types of immunity.

In another British study, H. J. B. Atkins (*Brit. Med. J.* **1,** *187* (*1958*)), 534 infants who had never been put to the breast were compared for the incidence of the common infectious diseases of childhood with 534 control infants who had nursed an unknown amount. The information was obtained by a questionnaire after a period of several years and was incidental to the main purpose of the study. No differences in incidence of infectious disease was found. The average age for measles was 32 to 35 months in this group of children and these data may not be comparable with those of Douglass.

Nordbring (*loc. cit.*) has reviewed the pertinent literature and points out that antibody levels for diphtheria, tetanus, antistreptolysin O, antistaphylolysin, streptococcal erythrogenic antitoxin, pertussis, typhoid H, measles, polio, influenza, mumps, toxoplasma, and univalent Rh antibodies are present in cord serum in relatively equal or larger amounts than in the maternal serum. Agglutinins to coliform organisms and typhoid O, as well as blood group isoagglutinins, are either absent from the cord serum or in much lower concentration than in the maternal serum. A study of human colostrum shows that it is the enteric agglutinins and the blood group agglutinins that are particularly prominent. In this study Nordbring demonstrated that between 40 and 50 per cent of colostrum samples showed a significant titer of antistreptolysin O or antistaphylolysin. This titer was lower than that in the mother's serum and virtually disappeared from the colostrum in three to five days. Significant titers of these antibodies were not observed in the infants ingesting colostrum from these mothers. K. E. Boorman, B. E. Dodd, and M. Gunther (*Arch. Dis. Child.* **33,** *24* (*1958*)) fed colostrum containing high titers of the blood group agglutinins anti-A and anti-B to 97 infants. This feeding was not accompanied by an increase in titer in the infant's serum. In a subsequent experiment the same authors fed serum containing very high titers of anti-D to 21 infants and were unable to demonstrate any appreciable antibody in their serum. It was calculated in both of these experiments that the sensitivity of the methods used would have detected the absorption of more than 0.3 or 0.67 per cent respectively of the two antibodies fed.

Nordbring (*Acta Paediat.* **46,** *569* (*1957*)) fed colostrum from recently immunized cows and pigs to nine premature infants. This colostrum was demonstrated to contain very high antibody titers to paratyphoid A antigen. These premature infants, who might be expected to absorb intact protein more easily than full term infants, failed to demonstrate any antibody titers to this organism in their serum, and it was estimated that absorption. if any, must have been less than 0.33 per cent.

A less specific approach to this subject has been taken by F. Hassan and M. Gunther (*Arch. Dis. Child.* **33,** *30* (*1958*)) who measured the serum proteins of infants at six months of age following breast, cow's milk, or mixed feeding by means of electrophoresis on filter paper. No differences in the serum gamma globulin concentrations among these three groups were found.

Although there is no immunologic evidence that antibodies present in human or bovine colostrum are absorbed to the extent of producing measurable antibody titers in the infant serum, there is sugges-

tive evidence that breast feeding may play some role in the development of immunity to at least one infectious disease in early life. The role played by non-specific factors has not been evaluated as extensively as that of specific antibodies.

BETA LIPOPROTEINS AND SERUM LIPIDS

It is impossible to predict whether or not a person will develop heart disease due to atherosclerosis. The presence of elevated cholesterol levels in the blood of atherosclerosis patients, and the occurrence of cholesterol in the atheromatous lesion, suggest the possible correlation between an elevated blood cholesterol level and the development of arteriosclerotic disease. It is well known that atheromatous lesions can be produced in experimental animals by including cholesterol in the ration.

Some clinicians and experimenters have questioned the value of blood cholesterol determinations in predicting the possible development of atherosclerosis. Experiments reviewed in *Nutrition Reviews* (**9,** *167 (1951)*) were designed to search for fractions of beta-lipoproteins, revealed in the ultracentrifuge, which correlated better with atherosclerosis than did the level of serum cholesterol. As to the nature of the blood beta-lipoprotein, J. L. Oncley, F. R. N. Gurd, and M. Melin (*J. Am. Chem. Soc.* **72,** *458 (1950)*) determined the composition of the beta-lipoprotein prepared in the ultracentrifuge following the low temperature alcohol precipitation of lipoprotein rich material. The polypeptide portion was 23 per cent and the lipid was 77 per cent. Direct analysis of the lipids gave 22.5 per cent ester cholesterol, 8.3 per cent free cholesterol, 39.1 per cent total esters, and 29.3 per cent phospholipid. The beta-lipoprotein carries 70 per cent of serum lipids and 75 per cent of the total cholesterol.

H. B. Jones *et al.* (*Am. J. Med.* **11,** *358 (1951)*) reported that the beta-lipoprotein fraction classed as S_f 12-20 correlated better with atherosclerosis than did serum cholesterol or the more dense, major portion of beta-lipoprotein material (S_f 0-11). An attempt was made to compare the prediction value of cholesterol versus beta-lipoprotein of S_f 12-20 and 20-100 in arteriosclerosis in a collaborative study involving several laboratories (J. W. Gofman *et al.*, *Circulation* **14,** *691 (1956)*). The determination of S_f 12-20 beta-lipoproteins in the ultracentrifuge was a difficult technique compared to cholesterol determination and would not be practicable in small laboratories.

Healthy persons and atherosclerotic patients do not have sharply delimited, unique patterns of serum lipids; lipid values characteristic of atherosclerosis may also be found in some apparently normal people, and vice versa. This is not surprising since atherosclerosis is widespread, requires a life-time in which to produce clinical effects, can be severe without being clinically evident, and is probably reversible.

Because many cases of severe atherosclerosis are difficult to diagnose on the basis of blood lipids alone, F. W. Fischer (*Klin. Wochnschr.* **35,** *373 (1957)*) has analyzed the lipid and lipoprotein spectrum of over 4500 atherosclerosis patients and 286 healthy persons using preparative and simple electrophoresis. The criteria used in selecting the control or "healthy" subjects were not described. One of the problems Fischer encountered in making his study was the difficulty in finding people who were free of atherosclerosis. A further problem in diagnosis was the determination of the extent of vessel changes in the patient. The newborn child served as an example of a type free of atherosclerosis. In addition,

severely undernourished patients with disturbance of fat absorption later at autopsy showed vessels almost completely free of atherosclerosis. Thus, in making a comparison of patients with normal individuals, the author was forced to accept a certain degree of atherosclerotic vessel change in normal subjects.

In view of these limitations and in accordance with the precedent set by D. P. Barr, *et al.* (*Am. J. Med.* **11,** *468 (1951)*) F. W. Fischer determined the lipoprotein values of 147 clinically healthy women, aged 18 to 34 years, and 101 men of the same age group. In the women's group 55 per cent had beta-lipoproteins less than 60 per cent of total lipoprotein, compared to only 27 per cent of the men. The relationship was reversed in the classification in which over 65 per cent of the lipoprotein was beta. The average per cent of beta-lipoprotein was 59 for the women and 64 for the men. In normal young women no beta-lipoprotein value exceeded 70 per cent of the total. About 13 per cent of the older atherosclerosis-free persons, and 90 per cent of atherosclerosis patients, exceeded the border number (70 per cent beta).

The relation of beta-lipoproteins to other lipids was studied by making about 1300 determinations of beta-cholesterol, 1800 of cholesterol, 1160 of phospholipid values and 200 of beta-phospholipid determined by preparative electrophoresis. Persons in the upper normal range of beta lipoprotein percentages had beta-cholesterol values averaging 80 per cent of the total cholesterol value. The highest normal cholesterol value in the group of healthy young women was 225 mg. per cent and the cholesterol/phospholipid ratio was 1.0. The average value of beta-cholesterol rose steadily with increasing beta-lipoprotein. In the middle of the beta-lipoprotein range (65 per cent of total) the beta-cholesterol values were also in the middle of the normal range.

In the pathologically elevated beta-lipoprotein range (75 relative per cent and above) the beta-cholesterol values were also elevated pathologically. In between there was a border range with a few overlapping values. For example, 30 per cent of persons with "normal" beta-lipoprotein values (65.5 to 70 per cent) showed slightly elevated beta-cholesterol values. Similarly, the 70.5 to 75 per cent beta-lipoprotein range included some 10 per cent of "normal" cholesterol values. In general there was a very significant relation between the beta-lipoprotein and beta-cholesterol values.

Total cholesterol showed a different relation as compared to its beta fraction. In the clinically most important group of beta-lipoproteins, ranging between 70 and 85 per cent of total lipoprotein, the average total cholesterol value was not elevated. In the normal beta-lipoprotein range elevated cholesterol values did not occur. In the pathological lipoprotein spectrum half of all cholesterol values did not exceed the upper value of the "normal" range. With slightly elevated lipoprotein spectra almost two thirds of the cholesterol values fell in the "normal" range, and with the greatest elevation of lipoprotein values F. W. Fischer found a "normal" cholesterol value for more than one third of all patients.

The phospholipid value was proportional to cholesterol in most atherosclerotic patients so that the cholesterol:phospholipid ratio did not change along with a pathologically altered lipoprotein pattern. The phospholipid was relatively low in 12 per cent of the investigated patients with a "normal" beta-lipoprotein value and a "normal" cholesterol value so that the quotient in these cases exceeded one. Normal values for the ratio were found in the pathological lipoprotein range in the majority of cases. No great significance was attached to the beta-cholesterol:beta-phospholipid ratio for the diagnosis of atherosclerosis. The values for both lipids rose and fell proportionately so that the average value 1.33 was similar in all lipoprotein groups.

Many investigators have studied the age and sex distribution of lipoproteins and lipids in normal people and patients. The

lack of unanimity in the results, according to F. W. Fischer, aroused the suspicion that factors other than eating habits played an essential role. For this reason he decided to reinvestigate the question using patients at the Hamburg hospital. The beta-lipoprotein and lipid data were studied in age groups arranged first by decades, secondly as larger groups from 31 to 70 years and 71 to 90 years, and finally, including all values between the ages of 31 to 90 years.

In 62 atherosclerotic patients between 31 and 40 years old no normal beta-lipoprotein values were found. Between the ages of 41 to 70 years there were from 2 to 6 per cent normal values. In the 71 to 80 year group one third of the men and one fourth of the women had normal values. Beyond 80 this percentage of normal values rose considerably with the men and remained essentially unchanged with the women. The percentage of normal values for men in the 71 to 90 year age group was ten times as high as that for men under 70 years of age. With women the increase was four times the value of the younger age group.

Corresponding to an increase in the number of normal beta-lipoprotein values in atherosclerosis patients in old age were significant decreases in average beta-lipoprotein values in later decades. Comparison of averages of men and women in single decades up to the fiftieth year showed no significant differences in beta-lipoproteins. In the sixth decade the average of the male patient group was significantly higher than that of the female group. In the following three decades the relationship was reversed so that the comparison of values in the collected 31 to 90 year group showed no significant sex differences.

The distribution of beta-cholesterol as determined by preparative electrophoresis was very similar to the distribution of the beta-lipoprotein determined by the oil red O technique in the different age and sex groups. The sex differences in beta-cholesterol values in single decades were similar to those of beta-lipoprotein in that the values for men were higher in the lower age groups and the values for women were higher in the later age groups.

Total cholesterol values which were definitely elevated in women in the sixth decade decreased in the seventh decade in both men and women. The decrease in the 71 to 90 year group, compared with the 31 to 70 year group, was highly significant and the values for men fell more than the values for women. The sex differences were not as pronounced in the single decades as they were for beta-lipoprotein.

The variation in blood phospholipid with age was similar to the cholesterol change. The average values decreased with advancing age, the decline being significant with men in the fifth to the sixth and seventh to eighth decade, and in women in the sixth to the eighth decade. The average phospholipid values for men rose from the fourth to the fifth decade and for women from the eighth to the ninth decade. The values for women were higher than those for men in the sixth to ninth decade. In contrast to the other lipids, the values for phospholipids in the collected group are higher in women than those for men. The cholesterol:phospholipid ratio showed no age displacement but the values for men were higher than those for women until the eighth decade.

The lowering of lipid and lipoprotein values which was observed in older patients represents, in the opinion of F. W. Fischer, a genuine decrease combined with a special selection of patients by survival of groups of people with primarily lower lipid and lipoprotein values.

In summary, the author observed that blood lipoproteins, as measured by paper electrophoresis, have a genuine significance in the pathogenesis of progressive atherosclerosis, the beta-lipoprotein being characteristically elevated. In contrast, the majority of the values for cholesterol, phospholipid and cholesterol:phospholipid ratios

were in the normal range. All lipoproteins and lipids showed characteristic sex differences and the average values decreased in the older age groups.

OBESITY AND LIVER IMPAIRMENT

The records of insurance companies indicate that the mortality rate of obese people, particularly males, is greater than that of those whose weights approach desirable values. In addition it has been noted that obese individuals have an increased incidence of diabetes, atherosclerosis, hypertension, and heart disease.

Recently J. O. Westwater and D. Fainer (*Gastroenterology* **34,** *686 (1958)*) reported studies in which they investigated liver function in obese patients. These investigators studied 18 selected obese patients. Each individual was carefully screened and all those who had evidence of frank liver disease, a history of previous jaundice or possible hepatitis, diabetes (or even a family history of diabetes) or more than a minimal intake of alcohol were not included in this study. Thus, none of the patients had a disease, or history of a disease, which in itself is known to cause liver damage, although one individual had cholecystectomy performed seven years previously for asymptomatic gall stones.

The patients in this study ranged from 28 to 69 years of age and had been obese from 6 to 30 years. They ranged from 25 to 110 per cent above desirable weights for their size. Of the 18 individuals studied, all except one were males. On each patient a variety of liver function tests were performed. These included the bromsulphalein retention test, glucose tolerance, cephalin flocculation, thymol turbidity, serum cholesterol level, in addition to a gall bladder series. The bromsulphalein retention test was measured in 14 patients. It was found to be abnormal in 13. An elevated bromsulphalein retention was the most consistent abnormality detected in this study.

It should be noted that the dye was given on a per kilogram basis so that the overweight patients received more dye than would persons of normal weight. However, these investigators indicated that the test is still valid under these conditions. Except for the glucose tolerance test, most of the other determinations were in the normal range for the majority of the patients, with only a few showing abnormal results. Eleven of the 17 individuals had a diabetic type of glucose tolerance.

Beside chemical tests for liver status, needle biopsies of the liver were performed on 12 patients. In eleven cases abnormal histological findings such as fatty infiltrations, foci of inflammatory cells, triaditis, or fibrosis were present in varying degrees. No correlation could be made between the histological findings and the degree of abnormality as shown by the bromsulphalein retention. However, in only one of the individuals tested were both the bromsulphalein test and the biopsy findings normal.

One of the patients during a year increased in weight from 265 to 284 pounds. During this time his bromsulphalein retention increased from 8.9 per cent to 11.3 per cent (less than 3.5 per cent retention was considered normal by this group). Contrarily, three patients had weight reductions to within 15 per cent of their desirable weight, and in these individuals there was improvement in liver function as noted by both the bromsulphalein test and glucose metabolism. However, in none of these patients was liver biopsy performed. This investigation demonstrates that abnormal liver function tests and changes in liver morphology are usually present in obese patients.

The cause of the aberration of the liver function of the obese patient is not known.

There is a suggestion, however, that these individuals derive most of their calories from non-protein sources and, consequently, may be on a poor protein intake. Such a diet has been known to produce fatty metamorphosis and cirrhosis of the liver in rats.

Thus, while a relative deficiency in lipotropic agents derived from protein may be one of the factors responsible for the etiology of liver impairment in the obese patient, it is not the sole cause. One individual studied had been on a high protein diet but did not show any improvement in liver function until he had lost considerable weight. Such a diet should have supplied adequate amounts of lipotropic substances. In the case of experimental animals it is known that obesity produced either by hypothalamic injury or in hereditary strains of mice has been accompanied by liver damage. Certain strains of geese are deliberately force-fed so that they become obese and at the same time produce a fatty liver which is to be used for *pâté de fois gras*. In the latter case the production of the fatty liver is apparently directly related to the obesity.

BIOCHEMICAL AND NUTRITIONAL STUDIES OF THE BANTU

The fortunate conjunction of a group of competent scientists interested in nutrition and a large fairly homogenous population available for study, should ideally lead to production of a wealth of valuable nutritional research. This situation appears to have taken place in South Africa where the Bantu population is in transition between primitive living conditions, and native diet, and urban life with an essentially Europeanized diet. Recently A. R. P. Walker (*Nutrition Reviews* **14,** *321 (1956)*) reviewed certain aspects of the nutrition of the South African Bantu from the points of view of (1) the effects of a rather specialized diet, (2) divergences from popular concepts of adequate nutrition and (3) relationships between the diet of the Bantu and his relative freedom from some of the so-called "diseases of civilization."

In a more extensive paper, Walker (*Ann. N. Y. Acad. Sci.* **69,** *989 (1958)*) has discussed these factors at greater length and in their relationship to certain unique biochemical findings. As was stated previously (A. R. P. Walker, *loc. cit.*) the diet of the rural Bantu consists of 50 to 90 per cent cereals (of which corn, kafir corn, and wheat are the main constituents), fairly large amounts of other vegetables (such as pumpkins, sweet potatoes and greens) and very small amounts (by European standards) of eggs, meat or fish. The diet is high (by accepted standards) in carbohydrate, fiber, vitamin A, phosphorus and iron and low in fat, animal protein, thiamine, riboflavin, vitamin D and calcium. Traditionally, it might be predicted that such a diet would produce a variety of common deficiency states which would be easily recognized in the population. That it does not, according to the author, should lead to reassessment of some presently well accepted ideas.

The average Bantu diet generally furnishes the adult with about 1 g. per kg. of total protein. However, it is so low in animal protein that a deficiency of certain amino acids should be apparent. With the exception of infants just after weaning age this has generally not been found to be true. Average weights do not appear to be subnormal and none of the usual symptoms is prevalent. For example, the lactation performance of Bantu mothers appears to be satisfactory in all respects. The breast-fed infant has a normal growth rate and does not suffer from any deficiency symptoms until after weaning. Chemical analysis reveals a higher average protein content (1.35 gram per cent) in milk of Bantu mothers than in that of American mothers

(1.06 gram per cent). Moreover, lactation failures are much less prevalent with the Bantu. Evidently the diet is satisfactory both in the amount of protein and the proportion of essential amino acids, at least for all but children between weaning and school age.

Liver dysfunction is often ascribed to a low protein intake or to specific amino acid deficiencies. However, although liver diseases are common among the Bantu (*Nutrition Reviews* **13,** *1 (1955)*), affecting more than two-thirds of the adults, they are not improved by a more adequate diet. In the opinion of the author, the liver conditions may have their origin in the damage suffered by the infant, rendering the organ more susceptible to disease in later life.

Another condition which has been ascribed to protein deficiency is gynecomastia, or enlargement of the male breasts. Although this condition is fairly common in young Bantu men, it differs in many respects from the same condition found following recovery from undernutrition. Among the Bantu, it is rarely accompanied by any other feminizing symptoms, either in appearance or function. Although the subjects themselves often state that the condition occurs in fathers or other close relatives, its etiology is still largely unknown and may again bear some relationship to nutritional injury in infancy.

Turning to the effect of the fat content of the Bantu diet, it has been observed by several workers that the low incidence of atherosclerosis (*Nutrition Reviews* **14,** *193 (1956)*) among these people correlates well with the very low fat content of their diet (about 15 per cent of total calories, largely of the polyunsaturated type found in cereals). Moreover, probably as a consequence of the large volumes of feces which are typical of the coarse cereal diet, fecal fat excretion is so high as to give an apparent fat absorption of about 50 per cent. Whether this indeed represents unabsorbed fat or a true excretion, the result is an even lower amount of lipid available for metabolism. This condition may well be the basis of the observations that serum lipid levels of Bantu apparently increase very little with age and that severe atherosclerosis leading to occlusions is very rare. The author does, however, consider that factors other than dietary fat may be important in these respects.

The physical condition of Bantu children and adults has been stated by several workers (as reported by Walker) to be superior to that of comparable white groups. It is possible that activity and physical fitness have considerable relationship to the blood lipid levels and atherosclerosis, but this subject needs further investigation. Of unknown significance is the observation that although the serum cholesterol levels are approximately the same in normal Bantu men and women, there is a tendency for many of the young women to become obese with a concomitant elevation of the serum cholesterol concentrations of about 25 per cent. This increase, however, is not accompanied by increased atherosclerosis.

The author discusses, as in the previous paper (*loc. cit.*), the fact that, despite a low vitamin D and calcium intake and an adverse calcium to phytate phosphorus ratio, there is no evidence of calcium deficiency in the rural Bantu. Rickets are uncommon, teeth are good, growth and attained height appear normal and no bone abnormalities are to be found. Moreover, it was observed that on the low-calcium high-fiber diet, absorption of calcium was higher and urinary excretion lower than among European groups, thus leading to increased retention of calcium. In general, these data cast doubt on the deleterious effects of phytate. They are in agreement with D. M. Hegsted, I. Moscosco and C. C. Collazas (*J. Nutrition* **46,** *181 (1952)*), on the adequacy of a lower intake of dietary calcium, especially for the adult.

The very high iron content of the Bantu diet leading to an individual consumption of as much as 200 mg. daily has been found to result in wide spread siderosis in the adults involving mainly the spleen, liver and bone marrow. Conversely, iron deficiency anemia is almost unknown in these people; it would be interesting to see if these large stores of iron could be utilized under conditions of low intake.

Two vitamins were included in the study which, from information previously available, would be expected to be deficient in the Bantu diet. The known role of corn in the etiology of pellagra (*Nutrition Reviews* **3,** *267 (1945)*; **4,** *334 (1946)*) would lead to the supposition that the Bantu should suffer from niacin deficiency. Actually the situation is not nearly so clear-cut. It was found that milk from Bantu mothers on a predominantly corn diet had an average of 70 micrograms per cent of niacin while that from mothers on a Europeanized diet had an average of 150 micrograms per cent despite the fact that both diets had approximately the same niacin content. The cause of this discrepancy is not known but may be related to the proposed niacin antagonist in corn (D. W. Woolley, *J. Biol. Chem.* **163,** *773 (1946)*). In any event, infants appeared to thrive equally well on both diets, both of which contain more niacin than the two thirds cow's-milk mixtures used among white populations.

The lack of infantile scurvy has been mentioned previously by Walker (*loc. cit.*) and is remarkable considering that the diet, in many cases, furnished less than 1 mg. of ascorbic acid per day. The high plasma ascorbic acid content of such infants has led to the suggestion that some synthesis may be promoted on this diet.

The conclusions to be reached from these findings include the following:

The gross composition of diets may be of great importance in determining minimum requirements of certain essential nutrients.

In many cases, notably calcium and protein, minimal requirements may be considerably lower than the generally recommended allowances.

The consideration that the biochemical differences found in primitive populations is abnormal as compared with the accepted norms, may be quite false, since with this reasoning the enviable low blood lipid values of the Bantu would be considered abnormal.

Studies of this sort are of value not only as they indicate specific nutritional requirements for human beings in general but also as they point up the different diseases prevalent in populations consuming quite different diets. It is to be hoped that the conditions which render the Bantu largely free from many of the so-called diseases of civilization do not inevitably lead to an increased incidence of those diseases to which the Bantu is now heir.

CALCIUM METABOLISM AND HYPERTHYROIDISM

There has been a re-awakening of interest in the effect of thyroid hormone on calcium metabolism in man. The disturbances in calcium and phosphorus metabolism in thyroid disease were subjected to careful scrutiny by J. C. Aub, W. Bauer, C. Heath and M. Ropes (*J. Clin. Invest.* **7,** *97 (1929)*). Using balance study techniques these workers established the occurrence of an excessive excretion of calcium both by the gastrointestinal tract and the kidneys. It was observed that "the calcium drain in exophthalmic goiter is even greater than in hyperparathyroidism." It is not surprising that long persisting hyperthyroidism may lead to depletion of bone minerals which

may be demonstrable by X-ray. The fact that this does not occur more frequently is attributable to the fact that treatment usually supervenes before clinically significant decalcification has occurred.

The action of thyroid hormone on bone salt metabolism received little additional study until the important contribution of S. M. Krane, G. L. Brownell, J. B. Stanbury and H. Corrigan (*J. Clin. Invest.* **35,** *874* (*1958*)). The turnover of bone minerals was examined by administering radioactive calcium (Ca^{45}) and measuring the total quantities of isotope excreted and the specific activities of the serum, urine and stool calcium at intervals up to three weeks. It was observed that the specific activity of the serum and urine calcium fell more rapidly in the four patients with thyrotoxicosis than it did in normal control subjects. The two patients with myxedema had a slower decline in the specific activity of calcium in serum and urine. The results were analyzed in terms of compartments in which Ca^{45} is distributed. The first of these represents the equilibration with extracellular fluid. The next compartment is attributed to the rapid reversible ion exchange on the surface of the bone crystals. Flow from the last compartment is attributable to new formation of bone crystals at the sites of active haversian systems where osteogenesis is occurring. The hyperthyroid individual showed an increase in the size of the calculated compartments and the rates of flow from the compartments; the latter finding is indicative of increased osteogenesis of bone calcium accretion. In view of the fact that these patients were usually in negative calcium balance, it is safe to assume that an accelerated rate of osteoclysis was also occurring.

The authors point out that the metabolic changes observed in hyperthyroidism resemble those found in hyperparathyroidism. One patient did have an elevated alkaline phosphatase, consistent with increased osteoblastic activity. Histologic evidence of increased turnover of bone salts in hyperthyroidism has been reported by R. H. Follis (*Bull. Johns Hopkins Hosp.* **92,** *305* (*1953*)).

The metabolic and histologic resemblance of the osseous changes of hyperthyroidism to hyperparathyroidism should lead one to anticipate alterations of the concentrations of calcium and phosphorus in the serum. In the past, elevations of serum calcium levels have been infrequently observed, three such cases having been described by E. Rose and R. S. Boles, Jr. (*M. Clin. North America* **37,** *1715* (*1953*)). Two recent and almost simultaneous reports are concerned with hypercalcemia in hyperthyroidism. The first of these clinical reports was by F. H. Epstein, L. R. Freedman and H. Levitin (*New Engl. J. Med.* **258,** *782* (*1958*)). A young man with thyrotoxicosis is described who had a serum calcium of 15.8 mg. per 100 ml. of serum. In addition, nephrocalcinosis, azotemia and impaired ability to concentrate his urine were present. Reversal of these changes followed antithyroid treatment.

The second report by C. R. Kleeman, S. Tuttle and S. H. Bassett (*J. Clin. Endocrinol.* **18,** *477* (*1958*)) presents long-term metabolic balances of a 58-year-old-man with hyperthyroidism and hypercalcemia. The degree of hypercalcemia found in this patient was less than the values reported by Epstein *et al.*, reaching only 13.6 mg. per 100 ml. of serum. A great increase in fecal and urinary calcium excretion was clearly evident and the negative calcium balance was not greatly influenced by changes in calcium intake. The negative calcium balance was clearly not related to negative caloric or nitrogen balances because the calcium losses persisted unabated when these abnormalities were corrected. When large amounts of phosphate were given during two five day periods there was a striking reduction of the urinary calcium, but fecal calcium excretion still exceeded intake. The total negative calcium balance during the 125 days of study was 39 g., which repre-

sented 3 to 4 per cent of the calcium content of his skeleton. Demineralization could not, however, be demonstrated radiologically.

A clinical remission followed treatment with antithyroid drugs and was associated with a decrease in fecal and urinary calcium excretions. Some time later a recurrence of hyperthyroidism occurred but hypercalcemia was not present and calcium balance was positive.

In commenting on the sporadic occurrence of hypercalcemia in hyperthyroidism, Kleeman, *et al.*, point out that this chemical disturbance is not related to the severity of hyperthyroidism. Evidence is cited that the parathyroid hormone may lower the renal clearance of calcium. In most cases of hyperthyroidism, a rise of the extracellular calcium as a result of the direct action of thyroid hormone on bone must inhibit parathyroid hormone release, thereby lowering serum calcium and increasing the renal clearance of calcium. Kleeman and co-workers would suggest that the failure of this homeostatic mechanism would lead to hypercalcemia in an occasional patient with hyperthyroidism.

In summary, it may be said that there is very convincing clinical and laboratory evidence that hyperthyroidism leads to a greatly augmented rate of bone mineral turnover which resembled hyperparathyroidism and the negative calcium balance which occurs may lead to demineralization of bone if allowed to continue. The sporadic occurrence of hypercalcemia may represent a failure to accommodate to an increase in the rate at which calcium enters the extracellular fluid. The hypothesis has been proposed that this may involve a failure of inhibition of normal parathyroid function.

NUTRITIONAL STATUS OF CUBAN CHILDREN

The Williams-Waterman Fund of the Research Corporation, New York City, has, for a number of years, taken an active interest in world-wide nutritional problems and has supplied some financial support necessary to gain knowledge of the nutritional health status of certain population groups. Through the Medical Committee of the *Fundacion de Investigaciones Medicas*, Havana, Cuba, a survey has been conducted of the nutritional status of the sixth grade school population of the island of Cuba (N. Jolliffe *et al.*, *J. Nutrition* **64**, *355 (1958)*).

The survey was conducted in the period January 16 to February 8, 1956. A sample from the sixth grade population (ages 11 to 13 years) was selected as representative of the entire population. At this age the special requirements of infants and young children (especially for protein) have passed, lifetime dietary patterns have been established, and the complications often present in adults, such as pregnancy and chronic disease, are at a minimum. Of the total sixth grade population of 41,883, 2171 subjects were examined; 1016 of these represented the urban population and 1155 represented the rural population, which is about the urban-rural distribution of the entire population of Cuba.

In terms of economic status, the survey excluded the wealthiest and the poorest but did include wide deviations from the average.

The 18 physicians who participated in the clinical examinations were selected on the basis of desire, interest, and ability to devote full time to the study. They participated in ten one-hour lectures given by one of the authors and directed toward familiarization with clinical signs of malnutrition.

Food consumption data were based on the estimates of the various foods available for human consumption as compiled by the Cuban *Institute Nacional de Reforma Economica*. Calculation of nutrients in the food supply was based largely on the data ob-

tained from nutrient composition studies of native Cuban foods conducted by the *Fundacion de Investigaciones Medicas*. According to these data rice constituted the largest single source of calories in the Cuban diet (22.2 per cent of the total). Sugar was next (17.5 per cent), followed by lards and oils (13.7 per cent), animal flesh (11.5 per cent), flour (8.8 per cent) and beans (5.6 per cent).

The dietary survey results, when compared with similar data from the United States, indicated that the Cuban diet was comparable with respect to ascorbic acid, carbohydrates and iron. All the other essential nutrients were substantially lower; the greatest deficiencies (less than 50 per cent of estimated intakes in United States) occurred in calcium, vitamin A, thiamine, and riboflavin.

When compared with the National Research Council allowances, the Cuban diet was found to be materially lower than U.S. figures for calcium, vitamin A, thiamine and riboflavin. The U. S. figures, it should be remembered, generally provide about a 50 per cent margin of safety exclusive of waste or cooking losses.

The clinical studies revealed that children in private schools were distinctly different from the public school population if one used weight-for-height as an estimate of normalcy. Normal, obese, and underweight subjects in the private schools were 70.6, 19.0 and 10.4 per cent, respectively, compared with 50.7, 5.6 and 43.7 per cent, respectively, in the public schools. If the 10.4 per cent underweight in the private school group can be considered representative of Cuban children with an adequate food supply, a large segment of the public school population can be presumed to suffer from malnutrition because of an inadequate food supply.

Underweight occurred somewhat more frequently in rural children than in urban children, while the reverse was true with respect to frequency of obesity.

Adolescent acne (acne vulgaris), if used as an index of onset of puberty, was in general correlated with adequacy of nutrient intake; this is in agreement with the well-known fact that under-nutrition often delays puberty.

Clinical signs by themselves are generally considered not to be sufficiently specific for given nutritional deficiencies. Despite this, surprisingly accurate predictions of laboratory findings could be made. This type of evidence was considered sufficiently adequate to lead the senior author to recommend that biochemical methods for vitamin A and carotene, ascorbic acid and riboflavin could be safely omitted in dietary surveys.

From the clinical and biochemical findings, the authors concluded that deficiencies of protein, vitamin A and ascorbic acid were practically non-existent in Cuba. Only 4 per cent of the children exhibited hemoglobin levels below 11 g. per 100 ml. of blood. This would imply adequacy of intake with respect to iron, folacin, protein, and vitamin B_{12}.

Data on body weights permitted the prediction that about one-third of the school children of this age group are not receiving sufficient calories to support normal weight gains.

Riboflavin deficiency, expressing itself as angular stomatitis or nasolabial seborrhea, was the most common clinically-manifest deficiency observed, and it occurred in 20 per cent of the population examined. The clinical data were supported by a low excretion of this nutrient in the urine (less than 100 micrograms of urinary riboflavin per gram of urinary creatinine in 30.8 per cent of the children).

Based on urinary excretions of thiamine and niacin metabolite (less than 50 micrograms of thiamine and less than 4 mg. of N-methyl-niacinamide per mg. of urinary creatinine), it was concluded that subclinical beriberi and pellegra are quite prevalent in Cuba even though there was no

clinical evidence of deficiencies of these nutrients.

Since rice is the most abundant single energy source available to the Cuban population, the authors emphasize that vitamin enrichment of this staple article of food would be a valuable contribution to the public health. If present estimates of food consumption remained stable and if rice were enriched to the level required for an enriched product in the United States, there would be a 60 per cent increase in riboflavin intake and 45, 30, and 13 per cent increases in the intake of thiamine, niacin, and iron, respectively.

It is of interest that the authors hardly comment on the relatively low supply of calcium available for consumption (400 mg. per person per day, or 38.5 per cent of comparable figures in United States). Skeletal deformities suggestive of healed rickets were found in 9.2 per cent of the private school children, in 9.3 per cent of the public school children, and 19.3 per cent of the urban Havana public school children.

The data on food availability indicate that 6.31 ounces of milk are available per capita daily for human consumption in Cuba and that this supplied approximately 40 per cent of the limiting amount of the riboflavin available. More significantly, the milk supplied about 60 per cent of the total available calcium.

The survey evidence indicates that the nutritional health of the Cuban population could be improved substantially by the simple expedient of rice enrichment. This is particularly significant when one considers that of the 5 million acres of arable land under cultivation in Cuba, 3.89 million acres are devoted to an export crop (sugar cane) which contributes little to the accessory-nutrient supply of the population.

WAR FOOD SHORTAGES AND DIABETES MELLITUS IN JAPAN

There appears to be a definite relationship between diabetes mellitus and obesity. On the other hand the most significant figures concerning the effect of widespread undernutrition on diabetes have been obtained from population groups subjected to poor nutritional intakes due to the various conditions associated with war, under which it is possible to determine the prevalence of a disease in a population group. Prevalence may be defined as the number of cases present in a given population at a given time, and it consists of the number of individuals having the disorder at the start of the period plus the number of new cases developing during the time of study.

Recently, Y. Goto, Y. Nakayama, and T. Yagi (*Diabetes* **7**, *136* (*1958*)) have reported on the prevalence of diabetes in patients who attended the Medical Clinic of the Tohoku University Hospital from 1936 through 1956. Beginning in 1941, rigid government regulations of food supplies were instituted. In the district serviced by this hospital, food shortages became severe beginning in 1942. At the end of the war in 1945, the food situation was extremely acute due to both decreased production and poor distribution. During this period death from starvation was not rare and lesser degrees of malnutrition were found frequently.

In the period 1936 to 1940 diabetic individuals averaged approximately 1.5 per cent of the out-patient population of the hospital. However, from 1941, when the food shortages were developing, there was a steady decrease in both the number and percentage of diabetic patients seen at the hospital. In 1947 the prevalence of diabetes in this group was minimum. This could have been due to an increased mortality, loss by evacuation among the existing diabetics, or a lower rate of occurrence of new cases.

The exact cause cannot be determined from the correlation data. The food supply of the country gradually improved beginning

in 1948, and by 1950 it was almost normal. However, the average caloric intake has only currently reached the suggested caloric requirement of the Japanese male.

In addition, the diabetic patients at this hospital were classified into two types. Type I consisted of those individuals with a mild type of the disease not requiring insulin for the control of the disorder. Type I individuals were mainly patients in the older age groups and the obese patients who were relatively insensitive to insulin. Type II diabetic patients consisted of the severe cases requiring insulin for the control of their disease. This group of patients was normally undernourished and usually sensitive to insulin. It was also noted that during years of poor nutritional intake the ratio of Type I to Type II diabetes declined from an average of approximately 1.8 per cent to a mean value of about 0.5 per cent.

Thus, under the severe food limitation in Japan during this time, many of the diabetic patients were actually being treated with a reduction in the caloric intake to the point of starvation, the pre-insulin era management for diabetes. From 1946 the caloric intake gradually increased and the average estimated caloric intake per man per day in an urban community in Japan rose from 1700 to about 2000 calories during this four-year period. The standard caloric requirement for an average Japanese urban male is considered to be about 2200 calories.

These investigators concluded that the World War II food shortage in Japan favorably affected the prevalence of diabetes mellitus in at least two ways: 1) In known diabetics the severity of the disease was reduced by the prolonged reduction of the caloric intake; 2) The development of clinically apparent diabetes was prevented in many instances, thus leading to a lower incidence of diabetes in the general population. From 1951 the ratio of diabetic individuals to the total population increased rapidly, attaining a peak of about 1.75 per cent.

It should be noted that in this work the prevalence of diabetes was related to the caloric consumption of the population. Whether or not there is a direct cause and effect relationship cannot definitely be determined. As these authors suggest, there may be many factors to account for a decrease in prevalence in diabetes during the years 1942 to 1948, including disruption of family groups, loss of property, as well as the uncertainty of the future and poor living conditions. While there is no doubt that all of these factors would increase the stress on an individual, they also include components which might influence the recording system (hospital visits) to a much greater extent than the actual prevalence of the disease. Yet these investigators noted that in other places, where food reduction occurred during the war but where the civilian populations were not actually participants in the war, similar reduction in the prevalence of diabetes occurred.

RUSSIAN RESEARCH ON EXPERIMENTAL ATHEROSCLEROSIS

Because of language and dissemination barriers, Russian medical research is not well-known in Europe and America. Consequently, Western readers often find this work strange and difficult to evaluate because of their lack of familiarity with its background and fashions. This sometimes leads the unwary or provincial reader to discount, ignore, or condemn good work which is merely foreign and strange.

A. L. Myasnikov has published an article, "Influence of Some Factors on Development of Experimental Cholesterol Atherosclerosis," from the Institute of Therapy of the Academy of Medical Sciences in Moscow (*Circulation* **17,** *99 (1958)*). This is

a 14-page summary of years of work done by the author and his collaborators, "submitted at the suggestion of Dr. Irving S. Wright, who became acquainted with the work of Professor Myasnikov during a visit to Moscow in October 1956."

The observations are chiefly on blood cholesterol levels and gross aortic lesions. The general experimental plan was to compare the amount of atherosclerosis in two groups of rabbits fed cholesterol (0.1 to 0.2 g. per kg. of weight daily for 100 days). One group served as control to the other, which received, in addition, the substance being tested. Blood cholesterol concentrations were measured every 10 to 15 days and the anatomic results were observed grossly on whole aortas laid open and stained with Sudan III. The findings are nicely shown by rows of photographs or sketches of the aortas with the lesions outlined.

Of numerous vitamins studied two, vitamin D_2 and ascorbic acid, had a pronounced effect. M. V. Bavina's studies showed that daily ingestion of 0.25 mg. of the former "drastically increased both the degree and rate of development of alimentary hypercholesteremia and the intensity of the aortic lipoidosis" as compared with that of control animals fed cholesterol only. The author dissociates the lesions from the muscle necrosis and calcinosis of the blood vessels, which occurred only occasionally, with the doses used in these experiments. Ascorbic acid (0.1 to 0.2 g. daily orally) was found to reduce the development of hypercholesteremia; in experiments reported by I. A. Myasnikova in 1947 it also diminished and retarded the development of "lipoidosis" in the rabbit aortas. Myasnikov gives evidence that ascorbic acid "stimulates" lipid metabolism, increases ketonemia, and clinically increases fecal cholesterol at the expense of blood cholesterol concentration.

"Neurotropic" drugs were tried by Y. T. Pushkar and associates, because of the possible connection between central nervous system disturbances and atherosclerosis. Phenobarbital (0.2 g.) and amphetamine (0.04 g.) were compared. Animals receiving the former were "semi-drowsy", had substantial decreases in blood pressure, and slight decreases in cholesterol and lecithin. As compared to those in the cholesterol-fed control animals, atherosclerotic lesions were markedly diminished in this group. On the contrary, the rabbits given amphetamine were excitable, had more labile blood pressures, and a number had increased arterial pressure. Animals of this group had greatly increased cholesterol levels (up to 2100 mg. per cent compared to a maximum of 800 mg. per cent in the cholesterol-fed group). Similarly, the aortic lipid deposits were greatly increased (and confluent throughout much of the aorta in several instances).

When amobarbital sodium or chloral hydrate were tested, cholesteremia was less than when cholesterol was fed alone. The reduction in aortic atherosclerosis was again striking and comparable to that with phenobarbital. Caffeine had no significant effect. Myasnikov concludes that with reduced excitability of the central nervous system, alimentary hypercholesteremia is sharply diminished and aortic lipoidosis is decreased. His work does not show how much of this is attributable to the cholesterol level and how much is due to the lowered blood pressure.

Also studied were 128 patients with "hypertonic disease" and atherosclerosis. The hypnotics amobarbital sodium, chloral hydrate and phenobarbital reduced the blood cholesterol levels, whereas the stimulants amphetamine and caffeine raised the levels after single doses; less effect was found after one or two weeks of daily administration. The author states, "our findings confirm the conclusion that the cholesterol content in the blood is controlled by the central nervous system."

When anticoagulants were studied in the usual rabbit experiments, heparin was found to reduce somewhat the hypercholesteremia of the cholesterol-fed control animals and

also the aortic lipid deposits. "Neodicoumarin" (evidently ethyl biscoumacetate) had no significant effect. The author infers that the heparin effect on cholesteremia is too slight to explain the anatomic results. He mentions the reduction of the serum beta-lipoprotein fraction by heparin, but does not go into the lipemia-clearing action of the latter.

Myasnikov reports on experiments carried out by N. N. Kipshidze on anoxia. Rabbits were fed cholesterol for six months. During the last four, they were kept for three to six hours daily in chambers with reduced oxygen content ("up to 12 per cent"). A control group without anoxia was fed cholesterol and another was made anoxic without cholesterol. The oxygen-starved, cholesterol-fed rabbits showed "much more" cholesterol in the blood than the cholesterol-fed control animals without anoxia. Photographs of five aortas of the former show almost completely confluent lipid deposits throughout, two or more times as extensive as in the cholesterol-fed group without anoxia. Similar changes are shown in the coronary arteries, together with areas of myocardial necrosis, usually subendocardial, and chiefly in the left ventricle and its papillary muscles. Anoxia without cholesterol produced no atherosclerosis; only "small proliferations with histiocytes occurred" in the heart.

Physical exercise on a treadmill to the point of "marked fatigue" was studied in other cholesterol-fed rabbits over a period of six months. These animals "showed a marked decrease of the blood cholesterol as compared with the group of rabbits that only received cholesterol." Atherosclerosis was reduced somewhat by exercise. However, focal necrosis was observed, occasionally extensive enough to be called an infarct. Additionally, several animals had myocardial scars, and one had a resulting aneurysm of the left ventricle. Coronary atherosclerosis and stenosis were pronounced in the rabbits with extensive necroses. The animals exercised without receiving cholesterol showed hypertrophy of the myocardium without other lesions. The author infers that, though coronary atherosclerosis and stenosis were less in the exercised animals, the functional demands of exercise were not met, leading to more necrosis than in the sedentary animals with narrower coronary arteries.

Myasnikov concludes: "this research has supplied us with convincing proof that it is quite possible to change the course of development of cholesterol atherosclerosis under experimental conditions."

Some questions may be raised about the dosages used. Thus, 250 mcg. of vitamin D_2 were given daily per rabbit, perhaps seven times the human therapeutic dose for rickets and $\frac{1}{15}$ that for a calcemic effect in the human adult.

Since the rabbit does not require vitamin D, its mode of action is a matter of speculation and one cannot infer whether the much lower quantities normally involved in human metabolism bear any relation at all to the problem of human atherosclerosis.

It should also be pointed out that ascorbic acid, phenobarbital and amphetamine were all given in very large doses.

The effects of amphetamine and hypnotics were ascribed to their action on the central nervous system. There is no mention of differences in dietary intake between the two groups, but the hyperactive rabbits presumably consumed considerably more food (and cholesterol with it) than the drowsy animals. This in itself should lead to higher blood cholesterol levels in the former, and perhaps accounts for the difference in incidence of atherosclerosis. In addition, one wonders whether the action of amphetamine and hypnotics on intestinal motility (through local effects on smooth muscle and the myenteric plexuses) may not markedly have altered intestinal absorption, thereby changing blood cholesterol levels and accounting partly for the increased atherosclerosis in the hyperactive animals and its diminution in the somnolent ones.

Another point, difficult to evaluate without reference to more detailed protocols, concerns the variation in controls. The plan and duration of the cholesterol feeding experiments were said to be similar throughout. Yet the mean blood cholesterol levels at 78 days (from inspection of the graphs) are about 200, 200, 500 and 800 mg. per cent in the four groups shown. Significance in the first instance is attached to a mean level of 650 mg. per cent in the group fed vitamin D_2. Although this is more than a threefold increase over the control values of 200, it is less than the 800 attained by the control animals in a different experiment. One wonders why the control groups varied so much and whether this casts any doubt on the validity of the vitamin D_2 experiment.

Nonetheless, Myasnikov's work is exceedingly interesting to Western readers because it concerns a number of factors in atherosclerosis which have received scanty attention from American and European investigators. The article, summarizing years of work by Myasnikov and his group, is replete with photographs and diagrams documenting this large mass of research in experimental pathology and therapeutics.

Scarcely any reference to the particular factors studied by Myasnikov appears in *Nutrition Reviews* in the past five years. One study did show that the addition of high doses of vitamin D to a choline-deficient diet resulted in an increased incidence of aortic sclerosis (57 per cent vs. 36), prevented by choline (*Nutrition Reviews* **13,** *189* (*1955*)).

Another paper, by C. D. deLangen and W. F. Donath (*Acta Med. Scandinav.* **156,** *317* (*1956*); *Nutrition Reviews* **16,** *159* (*1958*)) showed also that large doses of vitamin D increased the development of atherosclerosis and serum cholesterol levels in cholesterol-fed rabbits. These lesions were distinct from the intimal proliferations associated with the vitamin-induced medial necrosis. Similar findings were reported by E. Pfleiderer (*Virchows Archiv.* **284,** *154* (*1932*)).

In summary, Russian work on experimental atherosclerosis has shown that alimentary hypercholesteremia and atherosclerosis in the rabbit are reduced by the administration of ascorbic acid, hypnotics, heparin, and (slightly) by exercise. They are increased by vitamin D_2, amphetamine, and anoxia.

RICKETS AND FLUOROSIS

The discovery that the incidence of dental caries was diminished in regions where fluoride occurred naturally in the water has led to many controlled studies of the effect of fluoridation of public water supplies. Uniformly these studies have revealed that the reduction in tooth decay was due to the ingestion of a desirable level of fluorides during tooth development (*Nutrition Reviews* **9,** *21* (*1951*)). These and other studies have led to the conclusion that fluoride is an essential mineral nutrient necessary for the formation of healthy teeth with maximum caries resistance.

Many experiments with experimental animals and humans have contributed knowledge about the storage, excretion and mechanism of action of fluorides. In other experiments, the relative efficacy of different types of fluorine-containing compounds has been investigated (*Nutrition Reviews* **11,** *167*, *324* (*1953*); **14,** *197*, *230* (*1956*)).

The effect of larger quantities of fluoride sufficient to produce changes on the skeletal system, has not received as much attention as the influence of trace amounts of fluoride in the prevention of dental caries (*Nutrition Reviews* **4,** *145* (*1946*)). The amount of fluoride needed for effective caries prophylaxis is much less than the amount of fluoride which results in detectable skeletal changes. Under the conditions prevailing in

the Southern United States, the consumption of as much as 8 p.p.m. of fluoride in drinking water over 25-year periods did not result in adverse changes in the skeleton (N. C. Leone, *et al.*, *Public Health Reports* **69,** *925 (1954)*). In India, pathology of the skeletal system has been reported at lower levels of fluoride in the drinking water (C. G. Pandit, *et al.*, *Indian J. Med. Res.* **28,** *533 (1940)*). The subjects in the latter study had a high water consumption due to the high temperatures in the area and in addition were thought to have chronic insufficiencies of calories, calcium, vitamin C, and vitamin D.

Some researchers have noted thickening or osteosclerosis as the major defect resulting from fluorosis, whereas others believe that thinning or osteoporosis is the principal lesion. H. Kellner (*Arch. Exper. Path. u. Pharm.* **192,** *549 (1939)*) classified the fluoride effects in young dogs as similar to vitamin D deficiency rickets seen in this species.

L. F. Belanger, W. J. Visek, W. E. Lotz and C. L. Comar (*Am. J. Path.* **34,** *25 (1958)*) have reported a study of the pathogenesis of fluorosis as it affected the skeletal tissue of growing pigs. Castrated male pigs, six weeks old and weighing 65 lbs., were raised on a normal farm ration. They were then paired for the experiment, during which one of each pair received a ration containing 1,000 p.p.m. of sodium fluoride. The animals were fed for 30 days and then injected with 6 millicuries of $S^{35}O_4$. Pairs were killed at 10 minutes, 5 hours, 24 hours, 10 days, 30 days, 60 days and 90 days.

The ribs and heads of metatarsals were prepared for microscopic examination by fixing in formaldehyde, demineralizing in nitric acid, and sectioning in paraffin. Numerous stains were employed to facilitate the microscopic examination. Phase contrast microscopy was employed for some sections, and incineration to produce spodograms or ash patterns was used in others. Autoradiograms were prepared in order to determine the location of radiosulfate.

In similar experiments C. L. Comar, W. J. Visek, W. E. Lotz and J. H. Rust (*Am. J. Anat.* **92,** *361 (1953)*) had previously observed distortion in the usually linear arrangement of cartilage cells of the epiphyseal plate and encroachment of the zone by bone forming tissue. Osteoid or prebone was increased and the spicules (microscopic fragments of needle shaped bone) were decreased in size.

In the present series the outer diameter of the diaphysis (main shaft) of the bones was comparable in both groups, but the epiphysis (head) of fluorinated bones was beaded and double the size of the epiphysis of control animals at 90 days. The changes resembled those seen in the human rickets. The new deposits of bone in the fluorosed pigs were strikingly different from normal bone as observed microscopically. The osteoid (prebone) stained orange with the hematoxylin-phloxine B and orange G stain or green with the Masson treatment. Trabeculae (microscopic variously shaped bone units) stained red centrally and appeared to increase rapidly in thickness so that only a narrow border of yellow or green prebone was visible.

The fluorinated alveolar bone (forming the socket for the teeth) showed a decrease in acidophilia and the formation of bright globular bodies. The trabeculae later showed irregular growth and enlargement. The marrow cavities also were enlarged and blood vessels were distended. Bone matrix and cementum of fluorinated animals appeared fibrillar. The 60- and 90-day animals showed increased bone forming cells (osteoblasts) as well as more numerous bone absorbing cells (osteoclasts). Periosteal bone comprising the compact outer portion was affected more slowly. At the epiphyseal plate, where rapid growth occurs, the spicules became thinner and were more easily detached from the cartilage.

The cartilage head of the long bones hypertrophied progressively. This change was the result of an increased mitotic rate

in cartilage cells and was associated with immaturity combined with a decrease in matrix formation. The result was a cartilage that was very cellular and fragile and invaded by blood vessels. The epiphyseal plate was invaded by vascular, boneforming tissue from the marrow.

Tissues stained with toluidine blue may become pale blue or red-blue, the latter color being classed as metachromasia. The zone of mineralization of normal cartilage was weakly metachromatic but in the fluoride treated animals the color was a violet-blue, although unaltered in intensity. In contrast to metachromatically staining normal bone trabeculae, the fluorinated bone stain was a pale blue quality. The use of the periodic-acid-Schiff (PAS) stain leaves prebone unstained and imparts to normal bone a graded intensity of staining, increasing toward the center of the trabeculae. In contrast the PAS staining intensity decreased progressively in the bones of fluorinated animals. These two staining reactions indicated a decrease in the polysaccharide content in fluorosed bone.

Normal cartilage matrix appeared dark by phase contrast microscopy. The fluoride treated animals, however, showed fine bright globular material in the matrix between the rows of cartilage cells and large bright globular masses throughout newly formed bone trabeculae and in the marrow spaces near growing bone. These masses, which remained in spite of demineralizing treatment, were soluble in solutions of ammonium salts, a property characteristic of calcium fluoride.

Although demineralized tissues were not expected to yield much ash on incineration, a small amount of blue-white ash was seen in spodograms (ash patterns) of normal cartilage sections. Incinerated sections of fluorinated cartilage and bone showed more abundant ash similar to that seen in vitamin D deficiency rickets in children and strontium rickets in the rat. These ash deposits may have been organic salts of calcium rather than CaF_2.

In summary, the experiments demonstrated that a rachitic process, histologically comparable to that in vitamin D deficiency, was produced in the bones of pigs receiving fluoride in quantities of 1,000 p.p.m. of diet. The characteristic changes were decreased growth, decreased and imperfect mineralization, increased cartilage at the head of the long bones, diminished polysaccharide content of bone, overproduction of osteoid, increased acid resistant ash which was only partly calcium fluoride, and enlarged and malformed trabeculae. The authors point out those similarities to vitamin D deficiency rickets but caution that these changes may represent reactive, secondary changes. The experiments in this study delineate some of the tissue changes characteristic of fluorosis and show the need for experiments to determine the mechanism of the action of large intakes of fluoride.

While the current studies contribute fundamental knowledge about the influence of the ingestion of fluorides far out at the toxic end of the spectrum, they add little to our current knowledge of fluorides in human nutrition. The ingestion of 1000 p.p.m. in the diet for a maximum of 90 days is clearly in a different order of magnitude than the ingestion of the proper amount of fluoride for a lifetime to form healthy teeth with maximum caries resistance.

THE THERMOCHEMICAL EFFICIENCY OF GROWTH

In most nutrition experiments growth is measured in terms of weight gain, and efficiency of food utilization is estimated in terms of gain in weight per unit of food consumed. Such measurements do not take into account changes in body composition which, if they were known, might influence appreciably the interpretation of the weight

changes recorded and, even more so, the estimation of the efficiency of food utilization (*e.g.* the deposition of 1 g. of fat would represent a much greater energy accumulation than the deposition of 1 g. of protein).

It was with this in mind that J. Mayer and J. Vitale (*Am. J. Physiol.* **189,** *39 (1957)*) undertook to determine the "thermochemical efficiency" of growth in the rat. They defined thermochemical efficiency as *the ratio of calories deposited to calories ingested.* This differs from "energetic efficiency" in that it takes into account only the calories deposited in the body, not the calories used in performing work.

These researchers used weanling male albino rate in the three experiments they conducted. The diet for each experiment contained, in per cent: liver extract, 3; corn oil, 10; cod liver oil, 2; salt mixture, 4; and adequate amounts of the major water soluble vitamins. The remaining 81 per cent consisted of a mixture of lactalbumin and dextrose. The protein level of the diet was different in each experimental series. There was a low protein diet containing 10 per cent of lactalbumin, an adequate diet with 25 per cent of lactalbumin and a high protein diet with 60 per cent of lactalbumin. Food intakes were recorded daily and the rats were weighed every three days.

After 0, 3, 6, 9, 12, 18, 24, 30, 45 and 60 days, six rats representative of the entire population were fasted for four hours and killed with ether for carcass analysis. Moisture was determined by difference after drying the ground carcass for 48 hours at 100°C. over calcium chloride. Fat was determined by weighing the solvent free residue obtained after extracting the dried and ground carcass with petroleum ether. Nitrogen was determined by the Kjeldahl method on finely ground samples of the dried and defatted material. Protein was calculated by multiplying the nitrogen content by 6.25. Heat content was estimated from the protein and fat values, assuming that there were 5.7 and 9.5 calories per gram of protein and fat, respectively. Carbohydrate was considered to be negligible in relation to total body calories.

The results for the group fed the 25 per cent protein diet were considered as normal control values. Perhaps the most striking feature of the carcass analyses for this group was the very small variation in the protein content of the body throughout the 90 days following weaning. The average protein content was 16.9 per cent, with variations of only about plus or minus 1.5 per cent. In contrast, the moisture content dropped from 72 per cent at weaning to 56 per cent 90 days later. The fat content increased during this same period from about 5 to almost 23 per cent. The values for body composition differed somewhat for the low protein and high protein groups. In both of these the protein content tended to rise during the 90 day experimental period from 17 to 22 or 23 per cent, and there was a somewhat slower accumulation of body fat. The rates of gain of the 25 and 60 per cent protein groups were almost identical; that of the 10 per cent protein group was significantly less.

Another point of interest is that, although the rate of gain and the efficiency of food utilization (gain per unit of food consumed) of the control group fell off after about 21 days, the thermochemical efficiency (calories retained per calorie consumed) showed no drop from the value of 0.25 between the third and the fiftieth day of the experiment. It did decrease rather markedly, however, after this time when the rate of weight gain dropped sharply and the rate of increase in the fat content of the body also decreased. The thermochemical efficiency of rats on the high protein diet stayed constant until 111 days. In contrast, those on the low protein diet showed a drop in thermochemical efficiency earlier than either of the other groups.

It is evident from these results, as the authors point out, that body weight gain per unit of food consumed is a relatively poor index of caloric efficiency, particularly

in fairly long experiments. "An increase in weight of one gram between 60 and 90 days is equivalent in terms of energy stored to two to three times that increase at the beginning of postweaning growth." It is of importance, however, that the protein content of the body remained fairly constant throughout the first 90 days after weaning, an observation which indicates that weight gain is a fairly good index of protein deposition.

These experiments add to the general fund of reference data available for the rat and emphasize the importance of including changes in carcass analyses in estimating the efficiency of calorie utilization. From these observations it seems safe to conclude that, where measurements of calorie utilization are made without carcass analyses, experiments of short duration would provide more reliable information than longer term studies.

ASPIRIN AND MUSCLE METABOLISM

The demonstration that aspirin (acetylsalicylic acid) reduced hyperglycemia, glycosuria, and ketonuria in diabetes was the subject of a report by J. Reid, A. I. McDougall and M. M. Andrews (*Brit. Med. J.* **1,** *1071 (1957)*). The mechanism of this action is not known.

M. J. Smith and S. W. Jeffrey (*Biochem. J.* **64,** *589 (1956)*) found that uptake of glucose was somewhat inhibited when isolated rat diaphragm was incubated in phosphate buffered medium. P. J. Randle (*Nature* **178,** *983 (1956)*) noted that oxygen lack and compounds that interfere with the oxidative production of energy-rich phosphate promote the uptake of glucose by diaphragm in bicarbonate buffer but not in phosphate buffer. In view of the fact that salicylate interferes with this oxidative process, (B. W. Meade *Ann. Rheum. Dis.* **13,** *60 (1954)*), K. L. Manchester, P. J. Randle and G. H. Smith, (*Brit. Med. J.* **1,** *1028 (1958)*) investigated the effect of salicylate on the uptake of glucose by diaphragm in becarbonate buffered medium. In the review of the action of salicylate as related to pituitary-adrenal function C. H. Eades and J. S. King (*Int. Red. Med.* **169,** *642 (1956)*) noted that salicylate has a catabolic action similar to ACTH or cortisone. In addition the observation that C^{14}-labeled amino acids may be incorporated in the protein of isolated rat diaphragm (F. M. Sinex, J. McMullen and A. B. Hastings, *J. Biol. Chem.* **198,** *615 (1952)*) led Manchester, Randle and Smith to investigate the effect of salicylate on the incorporation of amino acids into the protein of isolated diaphragm.

Either bicarbonate or phosphate buffered medium was used. Glucose was added to give a concentration of 2.5 mg. per ml. The concentration of radioactive amino acids (One-C^{14}-glycine and uniformly labeled L-C^{14} glutamic and L-C^{14}-lysine) was about 0.1 micromole per ml., with a specific activity of 50 microcuries per mg. Sodium salicylate concentration was 5×10^{-3} Molar. Crystalline insulin concentration was 0.1 unit per ml.

Glucose uptake was calculated as the milligrams of glucose disappearing from the medium per gram of wet diaphragm per hour of incubation. Potassium loss was expressed as microequivalents of potassium added to the medium per gram of wet diaphragm per hour.

Thiosulfate, glucose and xylose spaces were determined by incubating whole diaphragm while attached to rib and vertebrae. The space was calculated as the ratio of the quantity in muscle to the quantity in the medium.

The uptake of C^{14} was determined in protein that had been twice precipitated by trichloracetic acid, washed with ethanol and ether, and dried under vacuum.

Salicylate (O-hydroxybenzoate) but not p-hydroxybenzoate added *in vitro* to a

medium buffered with bicarbonate increased the uptake of glucose by diaphragms. In phosphate buffered medium salicylate did not stimulate glucose uptake. Salicylate did not alter the total water or thiosulfate space (extracellular water) of diaphragm. Insulin addition did not alter the total water but reduced the thiosulfate space somewhat. Free glucose was entirely extracellular either in the presence or absence of insulin. D-xylose was present extracellularly in the absence of salicylate or insulin. When either salicylate or insulin was present, however, about 40 per cent of D-xylose acquired an intracellular distribution.

Salicylate led to greatly increased loss of potassium during incubation, in contrast to either p-hydroxybenzoate or insulin. Glutamic acid, C^{14}-labeled glycine, and lysine were incorporated into diaphragm protein. In the presence of salicylate the incorporation of the labeled amino acids was greatly reduced, but in the presence of insulin, incorporation was greatly increased.

The incorporation of amino acids into protein and the maintenance of intra-cellular potassium depend on the availability of high energy phosphate and the influence of salicylate on these processes supports the concept that it interferes with the oxidative production of high energy phosphate.

The fact that salicylate enhances glucose uptake suggests that metabolism is stimulated in peripheral tissues. Thus the reduction of glycosuria and hyperglycemia in diabetic patients treated with salicylate may be the result of increased glucose utilization by muscles. The fact that the volume distribution of D-xylose in the water of isolated diaphragm was increased by salicylate suggests that increased utilization of glucose was the result of increased rate of entry of glucose into the muscle cell. Since salicylate like insulin increased glucose uptake, expanded xylose space, but did not alter glucose space, K. L. Manchester, P. J. Randle and G. H. Smith concluded that increased glucose uptake included acceleration of the rate of glucose entry into the muscle cell.

These authors proposed that restraint of glucose entry into cells should be considered to be an active process since increased uptake of glucose resulted from the lack of oxygen or the inhibition of oxidation. The inhibiting effect of salicylate on incorporation of amino acids and retention of potassium is in keeping with the view that salicylate interferes with the production of high-energy phosphate. Insulin, on the other hand, enhances amino acid incorporation and potassium retention, therefore interference with the oxidative production of high-energy phosphate does not appear to be the mechanism whereby insulin promotes the uptake of glucose by muscle cell. Instead it may exert a specific interference with the availability of energy rich phosphate to the process restraining glucose entry.

K. L. Manchester, P. J. Randle and G. H. Smith indicate that more significance should be attached to the stimulating effect of salicylate on glucose uptake in bicarbonate buffer than to the lack of the effect in phosphate buffer. First, the bicarbonate buffer more closely resembles the ionic character of the extracellular fluid. Second, the phosphate buffer is unsuitable for incubation of tissues exhibiting a high rate of glycolysis, and, third, phosphate itself is able to influence glucose uptake.

In summary, the authors state that salicylate, like insulin, promotes the uptake of glucose by isolated rat diaphragm. The fact that salicylate seems to interfere with the incorporation of C^{14}-labeled amino acids into protein and increases the loss of potassium, while insulin has the reverse effect, suggests that insulin and salicylate promote glucose entry into cells by two different mechanisms, salicylate by inhibiting the production of energy-rich phosphate and insulin by limiting the availability of energy-rich phosphate to the process regulating glucose entry.

BEHAVIOR OF INSULIN LABELED WITH RADIOACTIVE IODINE

The use of isotopes has offered considerable information as to the mechanism of various chemical reactions and the distribution of substances in biological systems. With the present detection techniques it is possible in many cases to determine the isotope while having little or no determinable amounts of carrier present. This is particularly true for strong gamma emitters. Thus, the determination of iodinated albumin can be carried out in solutions too dilute to give the common protein tests.

The basis for all isotope work is that the tagged material does not behave differently in any manner except for its radioactivity than the original substance. In the case of iodinated proteins this need not always be true. It has been shown that the degree of iodination will influence the biological activity of many proteins. The role of I^{131}-labeled proteins in biology and medicine has been discussed recently (*Ann. New York Acad. Sci.* **73,** *3 (1957)*).

In studying the metabolism of insulin one of the techniques is to label it with I^{131}, making it radioactive. Again the assumption is made in all this work that the insulin's biological activity is not altered in the labeling process. One of the methods of measuring the tagged insulin in plasma is to precipitate the proteins with trichloroacetic acid and then to determine the radioactivity in this precipitate. In this case the assumption is made that all the activity found in the precipitate is due to the tagged insulin.

S. A. Berson and associates (*J. Clin. Invest.* **35,** *170 (1956)*) have reported that a variable portion of insulin is rendered physically different by the process involved in labeling it with I^{131}. They found that in using paper electrophoresis the unaltered insulin remained absorbed at the origin while the insulin which was altered in the labeling process moved at a rate which was similar to that of albumin. This was true whether or not additional plasma proteins were added to the sample. The altered fraction could account for as much as 25 per cent of the total radioactivity.

Recently G. W. Scott, T. E. Prout, J. A. Weaver and S. T. Asper (*Diabetes* **7,** *38 (1958)*) have reported studies in which they determined the amount of radioactive insulin present in blood after giving a dose of the tagged insulin using both the trichloroacetic acid precipitation method and paper electrophoresis. In addition, they determined the biological activity of the insulin in plasma employing a bioassay method dependent upon the glucose uptake of rat hemidiaphragms.

In a period of 30 to 70 minutes following the injection of tagged insulin it was found that the mean half life of insulin in the plasma was 67 minutes if one determined the activity in the trichloroacetic acid precipitates. But if one uses the radioactivity which remains absorbed at the origin in the electrophoretic strips (site for unaltered insulin) as an index, then the half life of the administered insulin becomes 24 minutes. Usually within 20 to 60 minutes following the administration of the tagged insulin the absolute difference between the activity found in the trichloroacetic acid precipitate and that remaining at the beginning of the electrophoretic strip remained quite constant. There appears to be no measurable biological decay of the remaining activity which migrates with the plasma proteins. It is believed that this recirculating altered fraction is responsible for the increased biological half life present in the trichloroacetic acid precipitates. The disappearance rate of the tagged insulin was not changed by simultaneous administration of large doses of crystalline zinc insulin, up to 100 units per kg. of body weight.

These investigators also determined by biological assay the insulin-like activity in the serum of rabbits given tagged insulin. They found that from 60 to 140 minutes

after injecting insulin there was a good correlation between the levels of insulin in the rabbit serum, as measured by bioassay, and the theoretical concentration of insulin I^{131}, as calculated from the previous electrophoretic study of the serum. At 140 minutes the insulin level had returned to the pre-injection levels as measured by bioassay, and the theoretical concentration of insulin as estimated by the electrophoretic technique, was also back to its pre-injection levels. But at this time, the insulin concentration as calculated from the trichloroacetic acid precipitates was persistently higher and showed a poor correlation with the biological assay of unlabeled insulin.

Thus the residual recirculating radioactivity which is equivalent to the altered insulin fraction is biologically inactive. Results which depend upon measurement of insulin activity in trichloroacetic acid precipitates of plasma, particularly after an hour or two following injection of the tagged material, would tend, according to these studies, to be erroneous.

These investigators also noted that a standard solution of tagged insulin had a smaller effect on the glucose uptake of the rat diaphragm than did an equivalent concentration of unlabeled insulin. From this work Scott and co-workers concluded that the labeled insulin had a decreased biological activity.

The insulin used in these studies was commercial iodinated crystalline zinc insulin, probably of bovine origin. The disappearance curves were determined after injecting it into rabbits. In using small doses of tagged protein the possibility of immuno-chemical reactions resulting from species differences should always be considered.

Scott and associates concluded that biological inactivation of insulin may occur during the tagging of insulin with I^{131} or *in vivo*. It is important to note that this inactivation may occur without the separation of the radioactive label from the protein. Thus, doubt is cast upon the use of insulin labeled with I^{131} as a reliable tag for insulin metabolism unless strict precautions are taken in the experimental procedure. By the use of electrophoretic techniques, or more careful iodination of the insulin, some of these difficulties may be eliminated.

EFFECT OF DIETARY FAT ON DEPOT FAT

The effect of dietary fat on the blood and organ fats has been a subject of considerable investigation for many years, especially since the development of the alkali isomerization technique, which permits convenient and rapid analysis of the polyunsaturated acids (*e.g.* V. H. Barki *et al.*, *Proc. Soc. Exp. Biol. Med.* **71,** *694 (1949)*; I. G. Rieckehoff, R. T. Holman and G. O. Burr, *Arch. Biochem.* **20,** *331 (1949)*; C. Widmer, Jr. and R. T. Holman, *Arch. Biochem.* **25,** *1 (1950)*; L. C. A. Nunn and I. Smedley-MacLean, *Biochem. J.* **32,** *2179 (1938)*).

The depot fats, however, have not received such attention; possibly the alterations in their composition have not been considered to be as interesting as have those of the other tissues. However, as one of the main destinations of dietary fat and one of the chief sources of the various fatty acids in the absence of dietary fat, they are actually of considerable interest and importance.

In a study of the effect of dietary fat on the subcutaneous fat of rats, F. Christensen, H. Dam and P. F. Engel (*Acta physiol. Scandinav.* **38,** *373 (1957)*) raised female weanling rats on a fat-free diet or the same diet with 28 per cent hydrogenated peanut oil. Samples of subcutaneous fat were taken for analysis until 131 days. Since the rats on the hydrogenated oil diet were in bad condition and losing weight at 98 days, the fat was removed from the diet and 16 mg. of linoleic acid per rat per day was substituted.

Analysis of the fat samples by measurement of conjugated diene absorption before and after alkali isomerization revealed that the linoleic acid content dropped precipitously in all animals during the first few days, especially in the animals on the fat-free diets, and then remained at a low level throughout the remaining time. In the case of the rats on the hydrogenated peanut oil-supplemented diets, there was a marked increase in conjugated diene before alkali isomerization despite the fact that the oil fed contained only 0.1 per cent of this substance. The authors considered that this conjugated acid might be the cause of the exaggerated ill-effect of the diet on the animals receiving the hydrogenated oil.

In a further study, Dam and Engel (*Acta physiol. Scandinav.* **42,** *28 (1958)*) studied the effect of 38, 89, 178 or 444 mg. per day of unhydrogenated peanut oil in the fat-free diet on groups of female weanling rats. The depot linoleic acid declined rapidly from an original content of 15 per cent to about 1 to 6 per cent in 80 days. At 183 days, when all rats were killed, percentages of dienoic and of tetraenoic acids of the depot fat were directly proportional to the peanut oil content of the diet. The trienoic acid remained constant at the low value of 0.3 per cent.

All animals showed some symptoms of essential fatty acid deficiency during the first few weeks, disappearing after 120 days in all but those on the fat free diet or the diet containing only 38 mg. of oil. It appears strange that any of the other groups should have shown signs of deficiency, since S. M. Greenberg and co-workers (*J. Nutrition* **41,** *473 (1950)*) have shown that 10 to 20 mg. per day of methyl linoleate was optimal for growth and presumably for prevention of deficiency symptoms in female rats. The present authors estimated by extrapolation that 1 g. of peanut oil (furnishing 200 mg. of linoleic acid) per day would suffice to maintain the dienoic acid content of the depot fats and, presumably, to prevent deficiency symptoms. This value, however, appears too high, especially for female rats, and a high depot linoleic acid content may not be an adequate measure of an optimal dietary content. It is unfortunate that male rats, which appear to have a much greater requirement for essential fatty acids, were not used in these experiments.

In the case of the liver fat, which was analyzed at the end of the experiment, trienoic acid, which is abnormally high in fat deficiency (J. F. Mead and W. H. Slaton, Jr., *J. Biol. Chem.* **219,** *705 (1956)*) decreased with increasing dietary peanut oil. Tetraenoic acid increased in the liver fat with increasing peanut oil, and the authors noted that the sum of trienoic and tetraenoic acids remained almost constant with these dietary changes.

Dam and Engel consider that these observations may provide evidence for a semi-hydrogenation of arachidonic acid to 5:8:11-eicosatrienoic acid in the fat-deficient animal. However, whether this is the correct explanation for its accumulation in this condition or whether, as is also possible, this acid is a dehydrogenation product of oleic acid, which could be attacked in the absence of linoleic acid (Nunn and Smedley-MacLean, *loc. cit.*; W. Montag, *et al.*, *J. Biol. Chem.* **227,** *53 (1957)*) can only be ascertained by further experiment. For these studies, the recently developed chromatographic methods of fatty acid analysis should prove invaluable.

CALORIC INTAKE AND NITROGEN BALANCE

During the past several years it has become apparent that the protein and amino acid requirements of an animal are closely interrelated with its caloric intake (*Nutrition Reviews* **13,** *71 (1955)*; **14,** *129, 232 (1956)*; **15,** *278 (1957)*). In particular, the experi-

ments of J. B. Allison and his co-workers have emphasized and clarified these relationships.

A review of these findings has recently appeared in the report of a conference on Protein Nutrition held by the New York Academy of Sciences on March 1 and 2, 1957 (J. B. Allison, *Ann. N. Y. Acad. Sci.* **69,** *1009* (*1958*)). Four relationships were discussed in the light of experiments carried out by the author and his associates.

1) On a constant nitrogen intake (3.82 g./day/M^2 body surface area) urinary nitrogen excretion of dogs varies inversely with caloric intake (H. L. Rosenthal and J. B. Allison, *J. Nutrition* **44,** *423* (*1951*)).

2) On a constant and adequate nitrogen intake, the rate of growth of tissues is primarily a function of caloric intake. On a protein-free diet, however, the liver protein of rats remained constant with decreasing caloric intake although the liver weight and lipid content decreased (Rosenthal and Allison, *J. Agr. Food Chem.* **4,** *792* (*1956*)). Thus, liver protein is apparently independent of caloric intake on a protein-free diet.

3) When dogs were fed varying amounts of diets containing different amounts of nitrogen in the form of egg protein, with adequate caloric intake or 50 per cent of the caloric requirements, the nitrogen balance index relating the change of nitrogen balance to nitrogen absorption (Allison, *Physiol. Rev.* **35,** *664* (*1955*)) was unity, indicating that all the nitrogen was retained (Allison, J. Anderson and R. D. Seeley, *Ann. N. Y. Acad. Sci.* **47,** *245* (*1946*)). When only 25 per cent of caloric requirements were furnished, however, this value was still unity at low nitrogen intakes but fell off with higher protein content, indicating more utilization of protein for caloric requirements under these conditions. When caloric requirements were partially filled with carbohydrate or lard, the nitrogen balance index returned to unity, approximately. Most authors, however, agree that carbohydrate has a specific nitrogen sparing effect not shown by fat, especially if the carbohydrate and protein are fed simultaneously.

4) When dogs were depleted of protein reserves on a protein free diet for 25 days, the filling of protein reserves could be assessed by analysis of nitrogen balances when different amounts of different protein were added to the diet. With 0.6 g. casein nitrogen per day per kg. of body weight in a diet containing 80 cal. per day per kg., nitrogen balance was established in about 23 days. The calculated nitrogen retention was 3.2 g. per kg. body weight. At 1.1 g. casein nitrogen in the same diet, about 10 g. was retained but the rate of re-establishment of equilibrium was about the same so that nitrogen balance was reached in a longer time with the higher protein reserves. When the daily caloric intake was increased to 144, nitrogen equilibrium was approached in 23 days, but 10 g. of protein had been added to the reserves.

It seems evident from these findings that an increase in protein content with constant caloric intake results in an increase in protein reserves at a constant rate of filling, while an increase in calories at the same level of protein results in a more rapid attainment of the same equilibrium point.

The nature of the protein is also important in these considerations, since, with the same levels of wheat gluten in these experiments, a much lower rate of attainment of equilibrium was observed. Addition of lysine to the wheat gluten brought the rate of filling of the protein reserves up to that observed with casein.

In summary, these experiments elucidate several relationships between nitrogen balance and caloric intake. In particular, they show that the filling of the protein reserves of a depleted animal is dependent on three parameters; (1) the nutritive value of the dietary protein (*e.g.* the ratio of the essential amino acids), (2) the nitrogen intake, and (3) the caloric intake.

NOTES

Metabolic Effects of Amino Acid Deficiency

The possibility that metabolic disturbances might occur if a protein containing an inadequate assortment of essential amino acids were ingested, has been studied by P. V. Simikov, Z. A. Kasperkaja and V. V. Kocegina (*Pediatrija (no. 7) 63 (1957)*). Weanling puppies were fed a diet complete in every respect, except that the normal tryptophan content of the protein was reduced by 60 per cent. The animals on this regimen gained only 78 per cent as much as the control animals. The plasma A/G ratio decreased as a result of diminished albumin concentration, and γ-globulin decreased by the fifth month while β-globulin increased. A decrease in the synthesis of hemoglobin was observed. Non-protein nitrogen rose.

Weanling rats were fed for three months on a tryptophan, methionine, and lysine deficient diet in which corn, gelatin, and a small amount of yeast, were the protein components. At the end of the time the content of blood globin and the urinary excretion of amylase, uric acid, and chloride were determined. The methionine deficiency was then partly corrected by the addition of cystine to the diet for a period of 12 days. This diet was then supplemented further with tryptophan for 14 days, and, finally, with lysine for eight days. At the end of each diet period the globin, amylase, uric acid, and chloride values were again determined. The excretion of amylase increased by about five times on the amino acid deficient diet.

Addition of L-cystine increased the excretion sharply, added L-tryptophan decreased it somewhat, but a combined supplement, including lysine, resulted in a marked decrease in the excretion of amylase. Tryptophan supplementation was followed by increased excretion of uric acid which, on further supplementation with lysine, was diminished. The excretion of chloride, which was increased following ingestion of L-cystine, was progressively restored to normal levels as tryptophan and lysine were added to the ration.

These observations suggest that evidence of a number of metabolic changes can be detected in rats and dogs ingesting diets that are somewhat deficient in amino acids. It would be highly desirable, however, to have information concerning the significance of these changes in experiments in which highly purified diets were used.

Appetite Stimulation in Children

An appraisal of the use of vitamins B_1 and B_{12} as supplements promoted for the stimulation of growth and appetite in children by the Committee on Nutrition of the American Academy of Pediatrics has appeared (*Pediatrics* **21**, *860 (1957)*). This committee has reviewed the pertinent literature and arrived at an opinion on this subject which should represent the current thinking of pediatricians. The committee points out that phases of irregular growth are normal for children, particularly during adolescence, and that a multiplicity of factors such as food habits, particular likes and dislikes, and cultural influences are all important in determining appetite, as well as the attractiveness of food preparation and the possibility of acute or chronic illness in the child.

This committee found no evidence that thiamine acts directly to increase appetite and interpreted the responses present in the literature as being associated with the treatment of manifest vitamin deficiency in animals.

Thirteen reports concerning the effect on growth of vitamin B_{12} administration to children are tabulated in detail. No convincing evidence for growth promoting effects of vitamin B_{12} was found. The prob-

lems of suitable controls, and the difficulties of working with human subjects, has made these studies particularly difficult, but it is clear that the administration of vitamin B_{12} does not encourage appetite so as to cause increased growth in children.

In conclusion, the committee suggests that "humility should govern the attitudes of manufacturers and physicians alike when it is urged that growth promoting substances be given to large groups of children."

Ferritin and Nutritional Edema

Ferritin is stored in the liver and spleen and ordinarily none is found in the blood. Under certain clinical conditions measurable amounts are detected in the blood; these include toxemia of pregnancy, hepatic cirrhosis, congestive heart failure and the nephrotic syndrome. In all of these conditions there is a retention of fluid and periods of oliguria (a diminution in the quantity of urine excreted). Ferritin has also been reported to produce a potent antidiuretic effect.

Recently, S. G. Srikantia (*Lancet* **1**, *667 (1958)*) investigated the possibility of ferritin being present in the blood of patients with edema due to protein and caloric undernutrition. He studied six children with kwashiorkor and two adults with nutritional edema. The assay of ferritin in plasmas of these individuals was determined using the rat mesoappendix test. On admission to the hospital, five of the six children, and both adults, had ferritin in their plasma. After 10 to 14 days of therapy, when there was no clinical edema, ferritin was detectable in the plasma in only two of the children and in neither of the adults. After one month of a high protein diet, none of the patients had ferritin present in the plasma. Thus, in the individuals where there was edema induced by poor nutrition, ferritin was present in the plasma, and when the edema disappeared following nutritional therapy the ferritin also disappeared.

While one might readily postulate a cause and effect relation between the presence of ferritin in plasma and the occurrence of nutritional edema, there are numerous other factors which must be considered (*e.g.* change in liver function and hemoglobin synthesis) before this relationship can be established.

Recent Nutrition Books

Dietetique Therapeutique. J. Tremolieres, A. Mosse & L. Delbes. Published by G. Doin & Cie, 8 place de l'Odeon, Paris. Pp. 555. Price, 6.500 fr.

Processed Plant Protein Foodstuffs. Aaron M. Altschul. Academic Press, Inc., 111 Fifth Ave., New York 3, N. Y. Pp. 955.

Frontiers in Science; A Survey. Edited by Edward Hutchings, Jr., with editorial commentaries by L. A. DuBridge, George W. Beadle, Harrison Brown, and Hunter Mead (all of California Institute of Technology). Basic Books, Inc., 59 Fourth Ave., New York 3, N. Y. Pp. 356. Price $6.00.

Procedures for the Testing of Food Additives: Second Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 1958, no. 144. Pp. 19. Price: 1/9, $.30, or Sw.fr. 1. Also published in French and Spanish.

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